HSB Project 7

Alkylanilines Class Study

Project Leader
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Background and Rationale
A subset of alkylanilines (2-ethylaniline, 2EA; 3-ethylaniline, 3EA, and 3,5-dimethylaniline, 3,5DMA) were nominated for toxicological characterization because of the potential for widespread human exposure, the limited availability of published toxicological data for this subclass of alkyl-substituted anilines, and their structural similarities to the known animal carcinogens 2,6-dimethylaniline (2,6DMA) and 2-methylaniline (2MA), which are known to form DNA adducts (Duan *et al.*, 2008; Skipper *et al.*, 2006).

Exposure to arylamines (a subclass of which is the alkylanilines) has been well documented in cigarette smokers (Bryant *et al.*, 1988; Luceri *et al.*, 1993), but also may occur from non-smoking related sources (Palmiotto et al. 2001). Smoking is associated with increased rates of bladder cancer (Cohen and Johansson 1992). The arylamines are believed to be the constituents of tobacco smoke that lead to the development of bladder cancer (Gan et al. 2004; Skipper *et al.*, 2003).

Alkylanilines are closely related structurally and are likely to be metabolized to *N*-hydroxylamines in vivo (Gorrod and Manson 1986). A fraction of the alkylanilines may also be metabolically transformed to DNA reactive quinone imines (Coleman *et al.*, 2000). Direct genotoxicity, as a byproduct of N-hydroxylamine or quinone imine formation, is the expected carcinogenic mode of action for these chemicals.

Based on the low level exposure of the general population to a large number of alkylanilines [3], their association with cancer in humans, and the observation that many of the alkylanilines are metabolized to potentially genotoxic species in humans, the NTP has proposed a class study of alkylanilines. The proposed studies are centered on determining the genotoxic mode of carcinogenic action of these chemicals. A tiered approach for these studies has been proposed as follows: Tier 1: *In vitro* mutagenicity studies with 14 members of the alkylaniline chemical class (2MA, 3MA, 4MA, 2,3DMA, 2,4DMA, 2,5DMA, 2,6DMA, 3,4DMA, 3,5DMA, 2EA, 3EA, 4EA, 2E6EA and 2M6EA); Tier 2: *In vivo* DNA adduct studies with select alkylanilines to evaluate correspondence to *in vitro* studies; Tier 3: Short-term carcinogenicity bioassays of select alkylanilines in genetically-altered mouse models; and finally Tier 4: Evaluation of the genetic determinants of alkylaniline genotoxicity. Tier 4 is the focus of the research concept presented here.

Genetic factors significantly influence both the formation of DNA reactive species from arylamines and the ultimate outcomes of exposure to this class of chemicals (Yu *et al.,* 2002). In particular, the genetically determined N-acetyltransferase 2 (NAT2) slow

acetylator, NAT1 rapid acetylator, and glutathione-s-transferase M1 (GSTM1)-null associated phenotypes have been correlated with increased risk of arylamine associated bladder cancer (Hein *et al.*, 2000; Marcus *et al.,* 2000). We propose studies to determine if susceptibility to the genotoxic effect of a subset of alkylanilines is consistent with genotypic variation at loci that determine arylamine bioactivation and detoxification. In short, our approach entails studies of genotoxicity in cultured hepatocytes from multiple inbred strains of mice followed by in silico, haplotype association mapping (Wang *et al.,* 2005).

Key Issues

In the human population, exposure to arylamines is associated with bladder cancer; however, we have proposed the use of hepatocytes to evaluate the genotoxic activity of these chemicals. We have selected hepatocytes because: (1) liver is a target of arylamine carcinogenesis in rodents (NTP, 1979); (2) hepatocytes exhibit the capacity to bioactivate arylamines (Danford, 1991); and (3) relatively high levels of DNA adducts have been observed in mouse liver following administration of select alkylanilines (Skipper *et al*., 2006). In addition to scientific justification for the use of hepatocytes, current protocols for culturing hepatocytes are more advanced than those for culturing urinary bladder epithelium.

In order to effectively evaluate the association between genetic loci that influence metabolic activation and genotoxicity, variation at these loci should be as diverse as possible. For this reason strain selection for the hepatocyte study will be based on a comparison of haplotypes at the following loci: *Cyp1a2*, *Nat1*, *Nat2* and *Gstm1*.

Approach and Specific Aims

These studies will employ cultured hepatocytes from inbred strains of mice that exhibit genetic diversity at loci known to influence the genotoxicity of arylamines (Yu *et al.,* 2002). Hepatocytes will be treated with equimolar doses of the select alkylanilines and DNA adduction and/or DNA damage (as measured by Comet assay) will be quantified**.** Quantitative measurements of genotoxicity will be used for haplotype association(s) mapping and identification of quantitative trait loci and candidate genes will be determined to test for the role metabolic activation in arylamine genotoxicity.

Specific Aim: Quantify the degree to which genetic variation influences the genotoxicity of select alkylanilines and identify alkylaniline genotoxicity quantitative trait loci.

Significance and Expected Outcome

These studies will provide insight into the degree to which genetic variation can influence intermediate phenotypes strongly associated with carcinogenic outcomes. By extension, such observations may lead to a better understanding and a more accurate estimate of population and personal cancer risk following exposure to alkylanilines. Potentially, the most intriguing outcome of these studies may be that genetic variation at loci that confer metabolic activation of the alkylanilines accounts for only a fraction of the variation in genotoxic effect and other genetic factors substantially modify the genotoxic hazard associated with these chemicals.

Current Activities

Initial studies to evaluate all 14 alkylanilines for genotoxic potency using an AS52 cell assay are currently being designed.

Future Plans

The proposed studies have the potential to identify currently unknown genetic loci that modify the carcinogenic risk associated with arylamine exposure and associations will need to be experimentally validated. Validation of novel risk modifying loci will be carried out using transgenic and/or knockout/knockin of targeted sequences in genetically altered mouse models.

References

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